Application Note

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LC/UV/MS Method Development and Optimization for Orforglipron Impurity Profiling

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Abstract

Pharmaceutical laboratories require practical LC/MS workflows to separate orforglipron from related impurities during method development and quality assessment. This application note demonstrates an Agilent LC/UV/MS solution using the Agilent 1290 Infinity II Bio LC System, an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column, and an Agilent InfinityLab Pro iQ Plus mass detector. The workflow enabled rapid method optimization, improved impurity resolution, and reproducible performance for confident orforglipron impurity profiling.

Introduction

Orforglipron is a small molecule, nonpeptide, orally administered glucagon-like peptide-1 receptor agonist (GLP-1 RA) being developed for the treatment of obesity and type-2 diabetes.[1] Unlike peptide-based GLP-1 therapies that require injection, orforglipron is designed for once-daily oral administration, making it a promising therapeutic candidate. As with other pharmaceutical compounds, comprehensive impurity characterization is essential to support product quality, stability assessment, and regulatory submissions.[2]

For laboratories developing impurity methods for emerging small-molecule GLP-1 receptor agonists, the key analytical challenge is to resolve the target compound from process-related and degradation impurities while maintaining consistent UV and MS response. Insufficient chromatographic resolution can affect impurity quantitation, obscure low-level components, and reduce confidence in product quality assessment. A practical screening workflow that evaluates column selectivity, mobile-phase composition, gradient conditions, and modifier chemistry can accelerate method development and help identify robust conditions for impurity profiling.

This study applies an Agilent LC/UV/MS workflow to support rapid orforglipron method optimization. The 1290 Infinity II Bio LC System provides high-performance chromatographic separation, the Poroshell 120 Phenyl-Hexyl column offers complementary selectivity for aromatic and hydrophobic structural features, and the Pro iQ Plus mass detector provides MS information to support impurity profiling. Together, this workflow enables laboratories to optimize separation conditions efficiently using accessible UV and single quadrupole MS detection.

Experimental

Reagents and chemicals

Orforglipron was obtained from MedChemExpress (Monmouth Junction, NJ, USA) and stored according to the manufacturer's instructions. DMSO and difluoroacetic acid (DFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC/MS-grade acetonitrile (ACN) and methanol (MeOH) were obtained from Agilent Technologies. Ultrapure water was collected from an in-house MilliporeSigma Milli-Q system (Billerica, MA, USA).

Analytical equipment

Agilent 1290 Infinity II Bio LC System including:

- Agilent 1290 Infinity II Bio High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Bio Multisampler (G7167B)
- Agilent 1290 Infinity II Thermostatted Column Compartment (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent InfinityLab Pro iQ Plus mass detector (G6170A)

Software

Agilent OpenLab CDS v2.8

Sample preparation

A stock solution of orforglipron was prepared at 1.0 mg/mL in DMSO. For LC/UV/MS analysis, the stock solution was diluted to 0.5 mg/mL in 30% ACN.

LC/UV/MS analysis

The LC and MS parameters are summarized in Tables 1 and 2.

Table 1. Liquid chromatography conditions.

Parameter	Value
LC	Agilent 1290 Infinity II Bio LC System
Columns	Column 1: Mixed-mode C18, 2.1 × 50 mm, 2.7 µm Column 2: InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1 × 50 mm, 1.9 µm Column 3: C18, 2.1 × 50 mm, 2.7 µm Column 4: C18, 2.1 × 50 mm, 1.9 µm Column 5: C18, 2.1 × 50 mm, 1.8 µm
Sample Thermostat	10 °C
Mobile Phase A	0.1% FA/DFA in water
Mobile Phase B	0.1% FA/DFA in acetonitrile/methanol
Gradient	Steeper gradient Time (min) %B 0.00 45 0.50 45 2.50 65 4.50 75 6.00 90 7.00 90 8.00 95 8.10 45 Shallow gradient Time (min) %B 0.00 20 1.00 20 5.00 35 10.00 50 12.00 70 14.00 80 16.00 80 17.00 20
Column Temperature	50 °C
Injection Volume	1 µL
Flow Rate	0.4 mL/min
DAD	280 nm

Results and discussion

Column selection

Figure 1 compares the chromatographic performance of five stationary phases evaluated for orforglipron analysis under identical chromatographic conditions. Key performance attributes included peak response, peak symmetry, column efficiency, and peak width. The Poroshell 120 Phenyl-Hexyl column produced the highest peak response, indicating enhanced analyte sensitivity, likely due to the phenyl-hexyl stationary phase chemistry that supports π-π and hydrophobic interactions with the aromatic and fluorinated moieties of orforglipron. This column also provided the lowest tailing factor, indicating highly symmetrical peak shape relative to the broader and more tailing peaks observed with the other columns. In addition, the Phenyl-Hexyl column gave the highest signal response, demonstrating better sample recovery and sharper peaks with minimal tailing. Columns 4 and 5 provided acceptable retention and peak shape but showed lower response and broader peaks. Although columns 1 and 3 enabled peak symmetry comparable with that of column 2, the sample recovery was relatively lower for the analyte under the evaluated conditions.

Table 2. Mass spectrometry acquisition parameters.

Parameter	Value
MS	Agilent InfinityLab Pro iQ Plus Mass Detector
Ion Source	Agilent Jet Stream ESI source
Polarity	Positive
Gas Temperature	200 °C
Drying Gas Flow	11 L/min
Nebulizer	35 psi
Sheath Gas Temperature	300 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	3,500 V
Nozzle Voltage	1,500
Fragmentor	135 V
Scan Type	Scan
Scan Time	500 ms
Data Storage	Profile
MS Spectrum Range	*m/z* 100–1,000

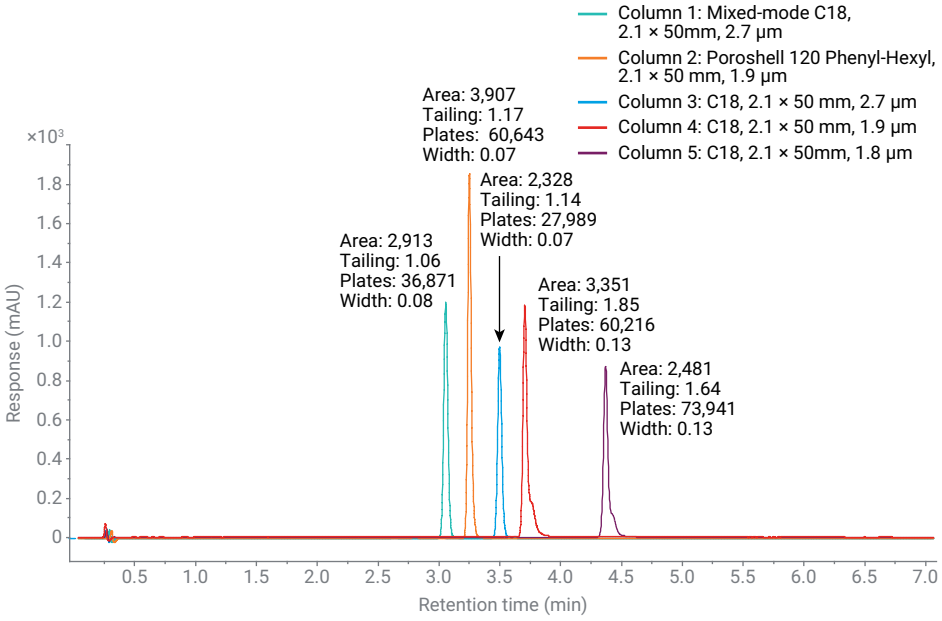

Figure 1. Comparison of chromatographic performance for orforglipron analysis using five stationary phases under identical LC conditions.

The impurity separation profiles further demonstrated the differences in column selectivity. The Poroshell 120 Phenyl-Hexyl column provided the most balanced separation, with impurity peaks well distributed across the chromatogram and baseline resolution achieved for critical peak pairs. Most impurity peaks were resolved with Rs > 1.5, supporting reliable peak assignment and minimizing potential overlap (Figure 2). In contrast, the other columns showed coelution or limited separation for closely eluting impurities, reducing their suitability for impurity profiling. Based on the combined peak performance and impurity resolution results, the Poroshell 120 Phenyl-Hexyl column was selected for further method development.

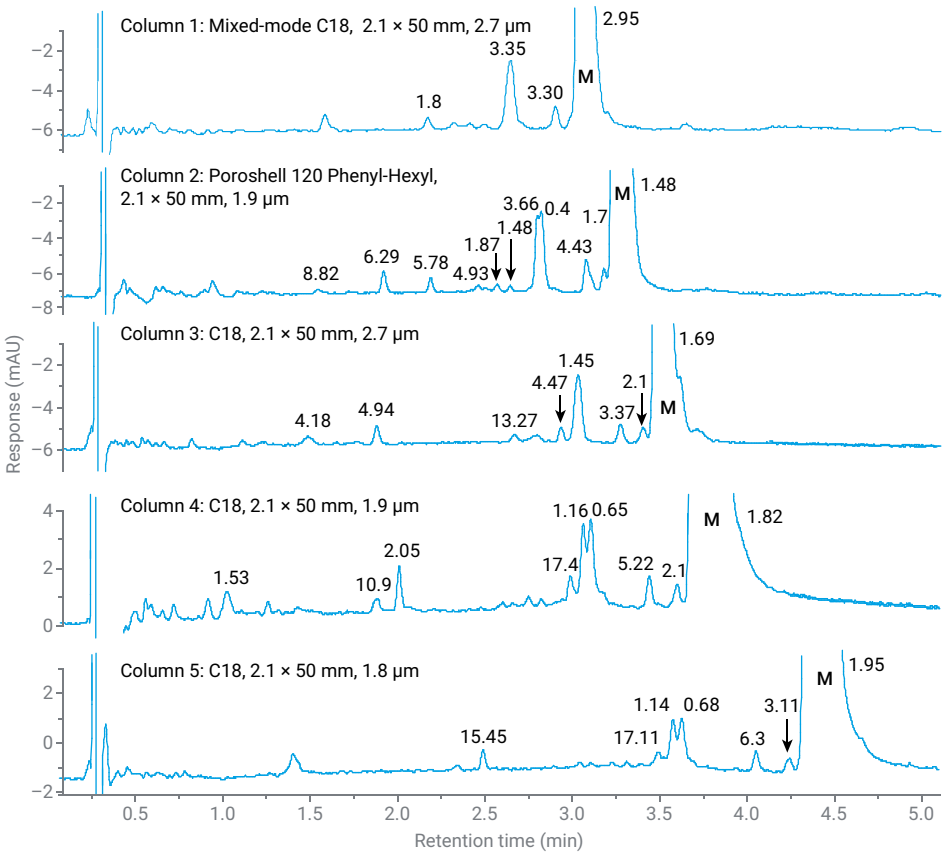

Figure 2. Expanded chromatographic view from Figure 1 showing separation of orforglipron from related impurities across the five stationary phases. M = orforglipron main peak. Annotated values in the chromatograms represent resolution values.

Organic mobile phase

Acetonitrile (ACN) and methanol (MeOH) were evaluated as organic mobile-phase components under otherwise identical chromatographic conditions. As shown in Figure 3, ACN produced a sharper and more symmetrical orforglipron peak, with lower baseline noise and higher signal response. In comparison, MeOH resulted in broader peaks and lower chromatographic efficiency. Based on these results, ACN was selected as the preferred organic modifier for subsequent method optimization.

Gradient profile

The gradient elution program was optimized to improve the separation of orforglipron-related impurities. Two gradient profiles were compared using the selected Poroshell 120 Phenyl-Hexyl column with ACN as the organic modifier. The steeper gradient, increasing from 45% B at 0 to 0.5 minutes to 90% B at 6.0 minutes, compressed impurity elution into a narrow retention window and resulted in closely spaced peaks. In contrast, the shallower gradient, increasing from 20% B at 1.0 minute to 80% B at 14.0 minutes, distributed impurity peaks over a broader retention range and improved peak spacing and selectivity (Figure 4). Therefore, the shallower gradient was selected for further optimization.

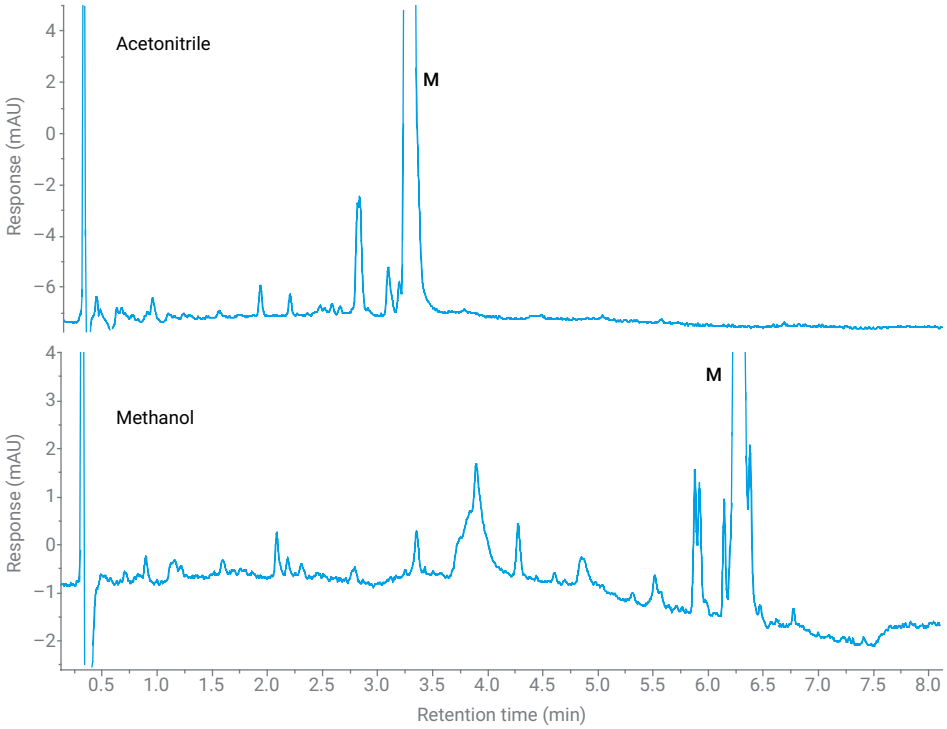

Figure 3. Effect of organic mobile-phase composition on orforglipron peak shape, response, and chromatographic efficiency using acetonitrile or methanol. M = orforglipron main peak.

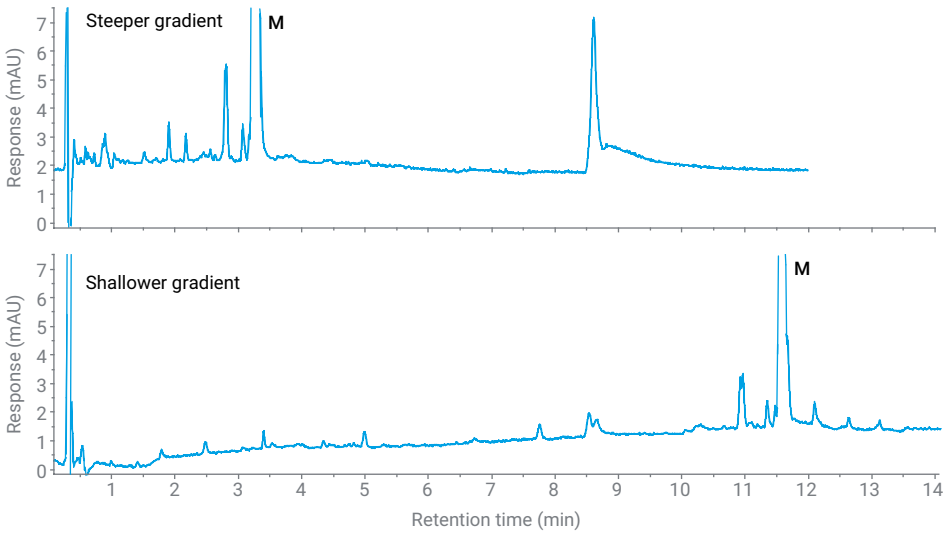

Figure 4. Effect of gradient profile on impurity peak distribution and chromatographic selectivity for orforglipron analysis. M = orforglipron main peak.

Mobile phase modifier

Two acidic modifiers, formic acid (FA) and difluoroacetic acid (DFA), were evaluated for their effects on chromatographic performance and MS response. As shown in Figure 5, DFA provided improved peak resolution and separation compared with FA. The total ion chromatogram (TIC) obtained with DFA showed a more defined MS response for the main orforglipron peak, whereas the FA condition produced a less distinct signal. Based on the combined improvements in chromatographic separation and mass spectrometric response, DFA was selected as the preferred mobile-phase modifier.

Method performance and reproducibility

Figure 6 shows the optimized LC/UV/MS method using the Poroshell 120 Phenyl-Hexyl column with a water/acetonitrile mobile phase containing 0.1% DFA and a shallow gradient program. Under these conditions, orforglipron was well resolved from related impurities and generated strong UV and MS responses. Replicate injections demonstrated excellent retention-time precision, with an average retention time of 11.528 minutes and an RSD of 0.008%. These results demonstrate that the Agilent workflow provides a practical and reproducible approach for method optimization, supporting confident impurity profiling with complementary chromatographic and MS information. Extracted ion chromatograms (EICs) were generated by selecting abundant mass-to-charge (*m/z*) signals corresponding to orforglipron and related impurities from the full-scan MS data.

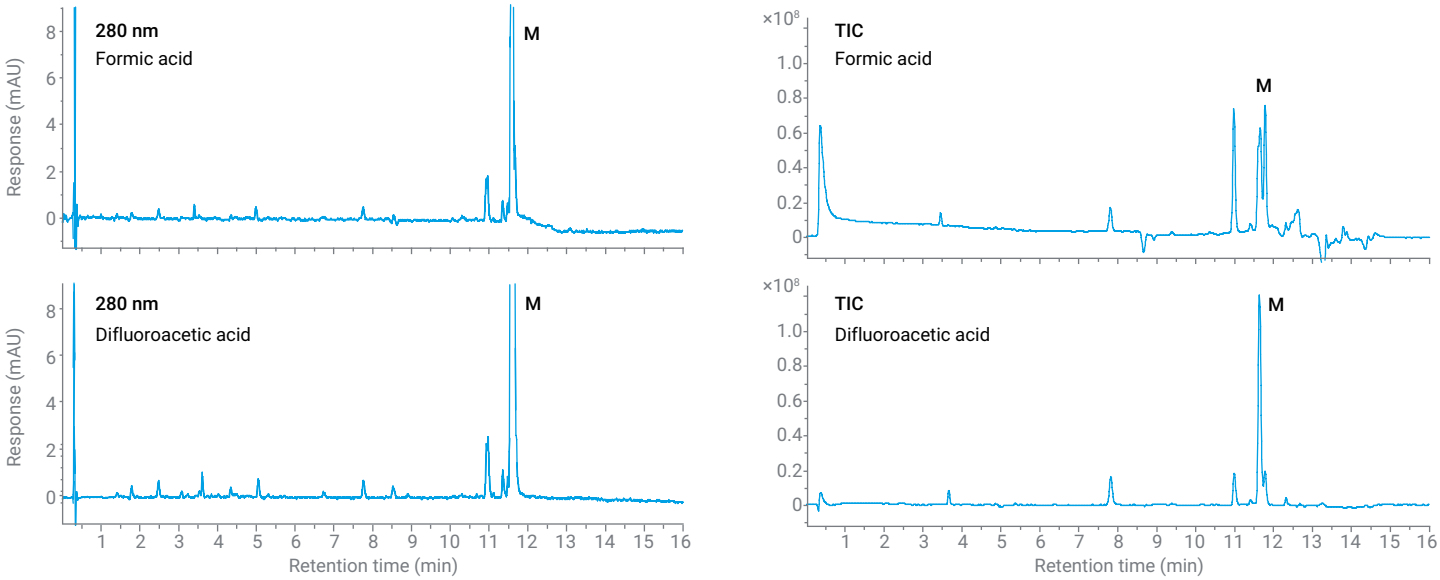

Figure 5. Comparison of formic acid and difluoroacetic acid as mobile-phase modifiers for UV and total ion chromatogram responses of orforglipron. M = orforglipron main peak.

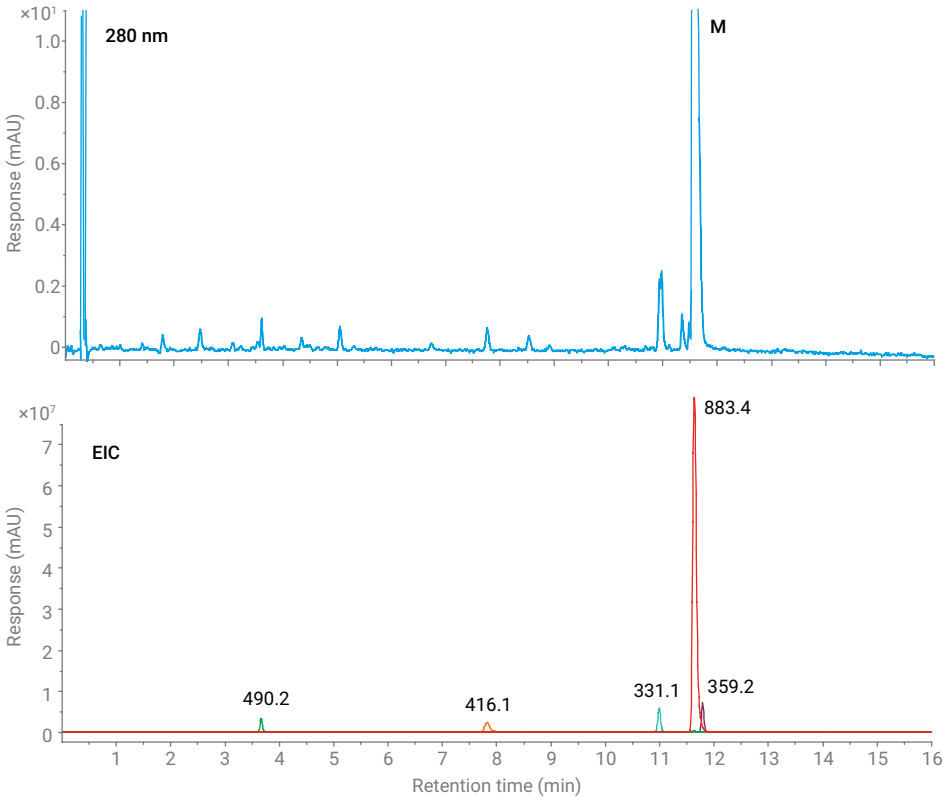

Figure 6. Optimized LC/UV/MS method showing UV detection at 280 nm and extracted ion chromatograms (EICs). M = orforglipron main peak.

Conclusion

Orforglipron impurity analysis requires reliable separation of related impurities with consistent UV and MS response. The Agilent LC/UV/MS workflow described in this study addresses this need by combining high-performance LC separation, Phenyl-Hexyl column selectivity, and single quadrupole MS detection. The optimized method improved impurity resolution, delivered reproducible retention times, and provided complementary UV/MS information, offering a practical solution for rapid orforglipron impurity profiling during method development and quality assessment.

References

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